Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1795–1799, March 1993
Cell Biology

Functionally distinct roles for glycosylation of α and β integrin chains in cell–matrix interactions

ROGER CHAMMAS*, SILVIO S. VEIGA*, LUIZ R. TRAVASSOS†, AND RICARDO R. BRENTANI*‡

*Ludwig Institute for Cancer Research, Rua Prof. Antonio Prudente, 109/4 01509, São Paulo, Brazil; and †Disciplina de Biologia Celular, Escola Paulista de Medicina, Rua Botucatu, 862/8 04023, São Paulo, Brazil

Communicated by Lloyd J. Old, October 2, 1992 (received for review March 2, 1992)

ABSTRACT **Laminin interaction with gp120/140, a B16-F10 laminin-binding protein immunologically related to α6β1 integrin, has been shown to be dependent on oligosaccharides from both ligand and receptor. Lectin analysis of gp120/140 led to the conclusion that this integrin is a sialoglycoprotein bearing mainly complex antennary structures. By means of exoglycosidase treatment, it was possible to identify α-galactosyl residues on the integrin α chain as the laminin-binding determinants. These residues are involved in cell adhesion to laminin. On the other hand, β-chain complex antennary structures, whose synthesis could be inhibited by swainsonine, were associated with cell spreading rather than cell adhesion. Thus, it was possible to modulate integrin-mediated cell adhesion and spreading through changes in the glycosylation state of integrin α and β chains.**

Cell–matrix interactions are mediated by integrin and non-integrin receptors (1). Integrins are heterodimeric glycoproteins consisting of two subunits, α and β. Combinations of two such polypeptides lead to the formation of different receptors, presenting different ligand specificities. Although the molecular basis of the integrin–ligand interaction is not completely understood, some experimental evidence suggests that posttranslational events are important determining factors (2).

In a previous report (3), we characterized the binding of laminin–nidogen complex (LN) to gp120/140, a LN-binding integrin from B16-F10 melanoma cells, as dependent on asparagine-linked oligosaccharides from both interactants. In the present study, we report on the further characterization of the oligosaccharide chains of affinity-purified gp120/140 based on its reactivity with a panel of lectins. We have evaluated the functional role of the sugar residues present in the integrin molecule in cell–matrix interactions. A suggestion is made of probable carbohydrate residues involved in adhesion to or spreading of B16-F10 cells on LN surfaces.

EXPERIMENTAL PROCEDURES

Cell Culture. Murine melanoma cells (B16-F10 line), a gift from M. Hendrix (University of Arizona), were maintained in tissue culture flasks with RPMI 1640 medium (Sigma), supplemented with 10% heat-inactivated fetal calf serum (Cultilab, Campinas, Brazil) and gentamicin sulfate (50 mg/ml) in a humidified incubator equilibrated with 5% CO_2/95% air at 37°C. Cells were harvested at subconfluence by rinsing culture flasks with phosphate-buffered saline (PBS)/EDTA. Cell treatment with swainsonine (SW; Sigma) was performed under culture conditions for 18 h, at a final concentration of 1 μg/ml. This SW concentration did not inhibit protein synthesis (as monitored by [^{3}H]leucine uptake) and displayed no effect on cell viability, as determined by trypan blue exclusion.

gp120/140 Characterization. gp120/140 was purified by affinity chromatography on LN-Sepharose columns (3). All the assays were done with Engelbreth–Holm–Swarm LN, purified as described (4). Affinity-purified gp120/140 was iodinated with Iodo-Gen (Pierce) (5) and then immunoprecipitated with GoH3 (anti-α6 rat monoclonal antibody, from Centraal Laboratorium van de Bloedtransfusiedienst, Amsterdam) as described (3). For immunodepletion studies, supernatants of immunoprecipitations with Rb3847 (an anti-β1-chain antiserum, provided by K. Yamada, National Institute for Dental Research, Bethesda, MD) were analyzed. Proteins were separated on either SDS/polyacrylamide gel (6) or two-dimensional SDS/polyacrylamide gel (7), transferred onto nitrocellulose filters, and analyzed by LN overlay assays and conventional Western blots. Glycosylation of affinity-purified LN-binding proteins was assessed in lectin or Western blots. Lectins used were either digoxigenin labeled [GNA (from *Galanthus nivalus*), DSA (from *Datura stramonium* agglutinin), SNA (from *Sambucus nigra* bark), MAA (*Maackia amurensis* agglutinin), PNA (peanut agglutinin) from Boehringer Mannheim] or biotin labeled [L-PHA (leukoagglutinin from *Phaseolus vulgaris*) and BSA (isolectin B4 from *Bandeiraea simplicifolia*), from Sigma]. Lectin binding was analyzed with suitable conjugates and chromogenic substrates.

Exoglycosidase Treatment of Wheat Germ Agglutinin (WGA)-Eluted Material. Sialic acids in the material eluted from a WGA-Sepharose column were removed with neuraminidase (EC 3.2.1.18; from *Arthrobacter ureafaciens*, purchased from Sigma), by incubating glycoproteins (≈100 μg) with the enzyme (0.05 unit) in 50 mM acetate buffer (pH 5.5) for 2 h at 37°C. Unmasked subterminal β-galactosyl residues were then excised with β-galactosidase (EC 3.2.1.23; from *Escherichia coli*, purchased from Sigma). This reaction was performed with 50 enzyme units per 100 μg of glycoprotein, in 20 mM phosphate buffer (pH 7.3) at 37°C for 18 h. Alternatively, glycoproteins were treated only with α-galactosidase (EC 3.2.1.22; from *Canavalia ensiformis*, from Sigma), by incubating 100 μg of glycoprotein with 1 unit of the enzyme in acetate buffer (pH 6.0) at 37°C for 18 h.

Deglycosylation reactions were monitored with suitable lectins or antibodies. Lectins used routinely were from *Sambucus nigra* bark for neuraminidase treatment, DSA for β-galactosidase treatment, and BSA for α-galactosidase treatment. α-Galactosidase treatment was further monitored with affinity-purified anti-α-galactosyl IgG (8) in Western blots. LN-binding activity of deglycosylated gp120/140 was tested in LN overlay assays essentially as described (3).

Cell Adhesion and Spreading Assays. Adhesion assays were done as described (3). The role of α-galactosyl residues in B16-F10 adhesion to LN was studied by incubating cells with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LN, laminin–nidogen complex; FN, fibronectin; L-PHA, leukoagglutinin from *Phaseolus vulgaris*; SW, swainsonine; GNA, *Galanthus nivalus* agglutinin; MAA, *Maackia amurensis* agglutinin; BSA, isolectin B4 from *Bandeiraea simplicifolia*; WGA, wheat germ agglutinin.

‡To whom reprint requests should be addressed.

either human anti-α-galactosyl IgG (10 μg/ml) or normal human IgG as a control (10 μg/ml) for 30 min at 4°C and then plating them on LN or albumin-coated surfaces (10 μg per well) in a conventional cell adhesion assay.

SW effect on cell spreading was studied by plating control and treated cells on LN- or fibronectin (FN)-coated surfaces (10 and 5 μg/ml, respectively). After 60 min, adhered cells were fixed with 2% PBS-buffered formaldehyde and analyzed in an inverted tissue culture microscope (Olympus, Tokyo). Random fields were photographed, and morphometric measurements were done to assess cell spreading and analyzed by Student's *t* test.

Cell ELISAs. Control or SW-treated cells (10^4 cells) were plated on 96-well tissue culture plates for 18 h, fixed with 2% glutaraldehyde (nonpermeabilizing fixation), and then processed (9). Biotin-labeled L-PHA (for SW treatment control), GoH3 (anti-α6 chain), and Rb3847 (anti-β1 chain) were used as probes and developed with appropriate conjugates. Values obtained using an EIA reader (Bio-Rad) were analyzed by Student's *t* test.

RESULTS AND DISCUSSION

Biochemical Characterization of gp120/140 and Its Glycans. We had characterized gp120/140 as immunologically related to the α6β1 integrin subfamily (3). Fig. 1*A* depicts an immunodepletion assay of B16-F10 cell extract using anti-β1-chain polyclonal antiserum (Rb3847). Immunodepletion was monitored with Western blots of supernatants from consecutive immunoprecipitations (Fig. 1*A1*) either with normal serum (lanes A–C) or with Rb3847 (lanes D–F) and then analyzed in LN overlay assays (Fig. 1*A2*). A decrease in the content of β1-chain polypeptides in the supernatants studied (Fig. 1*A1*, lanes D–F) was accompanied by a decrease in laminin-binding activity (Fig. 1*A2*, lanes D–F)—i.e., a decrease in gp120/140 content—characterizing gp120/140 as an αβ1 complex.

Fig. 1*B* depicts the immunoprecipitation profile with GoH3 from both metabolically labeled B16-F10 cells (lane A, whole cell extract; lane B, immunoprecipitated material) and ^{125}I-labeled affinity-purified gp120/140 (lane C). Since this material was analyzed under reducing conditions, the band presenting the smaller molecular mass is likely to be the α6 chain and the larger molecular mass band represents the β1-chain polypeptide. LN leakage from the affinity column was also observed, explaining the diffuse bands present in lane C. Thus, gp120/140 is immunologically related to the α6β1 integrin subfamily.

Glycosylation of affinity-purified gp120/140 was analyzed with lectins or antibodies used as probes for defined oligosaccharide chains (Fig. 1*C*). Lectins were MAA (lane A), GNA (lane B), L-PHA (lane C), and BSA (lane D). Affinity-purified anti-α-galactosyl IgG was also used (lane E).

MAA recognizes sialic acids linked α2–3 to galactose (10); reactivity with gp120/140 characterized it as a sialoglycoprotein. GNA recognizes terminal D-mannose, especially in the αMan(1–3)Man structure (11). gp120/140 was not recognized by GNA (Fig. 1*C*, lane B), which suggests that no terminal mannose residues are present in this molecule. The absence of either N-linked high mannose or hybrid-type oligosaccharide structures in gp120/140 indicated that this laminin-binding integrin bears mainly complex antennary structures.

L-PHA requires galactose and the β1–6-linked antenna in tri- or tetraantennary structures for high-affinity binding (12). gp120/140 has such a structure as shown by reactivity with this lectin (Fig. 1*C*, lane C). gp120/140 also has terminal α-galactosyl residues, assessed by reaction with BSA or reactivity with affinity-purified anti-α-galactosyl residues (Fig. 1*C*, lanes D and E, respectively).

Dennis and colleagues (13) compared the glycopeptides present in metastatic and nonmetastatic variants of a murine lymphoreticular tumor cell line and found that, in this model, the metastatic cells had both sialylated and nonsialylated β1–6-branched triantennary poly-*N*-acetyllactosamine structures. These structures, also expressed in gp120/140, inhibited adhesion of the lymphoreticular cells to LN, but not to FN or type IV collagen. The role of the glycosylation pattern present in gp120/140 was studied by exoglycosidase treatment and inhibition of the β1–6-branched oligosaccharide synthesis as discussed below.

Participation of gp120/140 Terminal Sugar Residues in LN Binding. The role of gp120/140 terminal sugar residues in LN binding was assessed by treating WGA-eluted melanoma

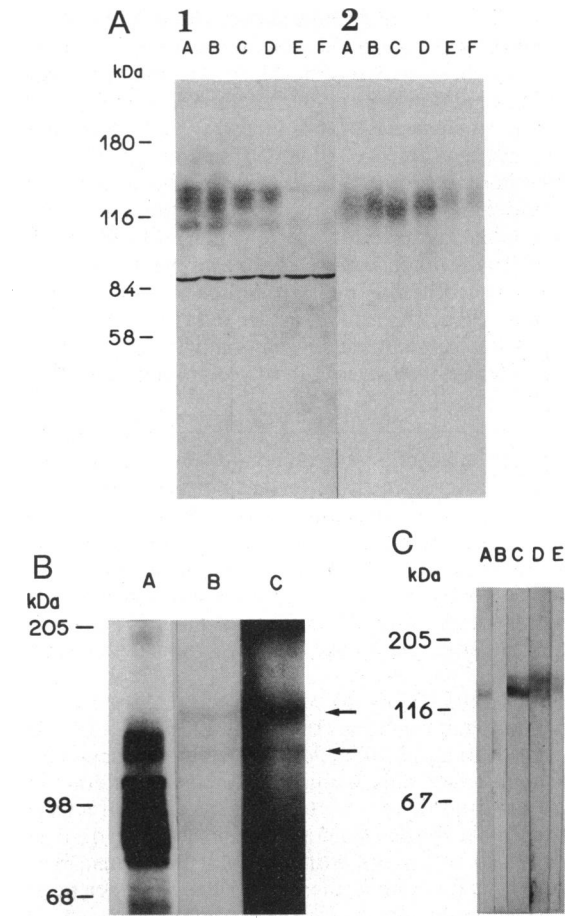

FIG. 1. Biochemical nature of gp120/140 and its oligosaccharides. (*A*) Immunodepletion of B16-F10 cell extracts. Supernatants of first, second, and fourth consecutive immunoprecipitations with either normal serum (lanes A–C) or anti-β1-chain antiserum (lanes D–F) were separated on SDS/polyacrylamide gel under nonreducing conditions, transferred onto nitrocellulose, and then analyzed for the presence of β1 chain (*1*) or LN-binding activity (*2*). Decrease of LN-binding activity after depletion of β1-chain polypeptides indicated the integrin nature of gp120/140. (*B*) Immunoprecipitation with GoH3. Autoradiograph of B16-F10 whole cell extract (lane A), immunoprecipitated material from this extract (lane B), and immunoprecipitated material from affinity-purified gp120/140 (lane C) analyzed under reducing conditions. Arrows indicate α6 and β1 chains, which constitute gp120/140. (*C*) gp120/140 oligosaccharides. Terminal sialic acids in affinity-purified gp120/140 were recognized by MAA (lane A). gp120/140 was not recognized by GNA (lane B); thus, no terminal mannose is present in this LN-binding integrin. L-PHA reactivity (lane C) indicated the presence of tri- or tetraantennary structures in gp120/140. BSA and anti-α-galactosyl reactivity (lanes D and E, respectively) indicated the presence of terminal α-galactosyl residues in gp120/140. Thus, analysis of gp120/140 glycans allowed us to classify it as a sialoglycoprotein, bearing mainly complex antennary structures and presenting terminal α-galactosides.

extracts with neuraminidase, neuraminidase and β-galactosidase, or α-galactosidase. As depicted in Fig. 2*A* and discussed elsewhere (3), under nonreducing conditions, the LN-binding protein (lane A) comigrates with the β1 integrin chain (lane E).

Neuraminidase-treated gp120/140 was still able to bind laminin (Fig. 2*A*, lane B); a band shift of ≈15 kDa was observed in the LN-binding polypeptide, whereas the molecular mass of the β1 chain varied to a lesser extent (5 kDa), as shown (lane F). Variation of molecular mass, as well as lectin probing (data not shown), ascertained the activity of the enzyme. The differential sensitivity to neuraminidase of gp120/140 and the β1-chain polypeptide suggested that the laminin-binding polypeptide is not the β1 chain.

Both chains of gp120/140 are highly sialylated. Sialylation of gp120/140 is the same order of magnitude as that ascribed to VLA-6 from murine platelets (14). Nevertheless, highly sialylated cell-surface glycoproteins are a common feature from both murine and human melanoma cells (15). Despite the high content of sialic acid residues in gp120/140, they did not affect the interaction between LN and its receptor.

The role of sialic acids in cell–matrix interactions has been extensively discussed; sialidase treatment of colon carcinoma cell lines had no effect on their adhesion to laminin (16). Removal of polysialic acid chains from cell-surface glycoproteins of F11 cells enhanced their adhesion to LN (17), suggesting that sugar residues, normally masked by sialic acid, may participate in interactions with LN. In the murine melanoma model, the metastatic potential correlates with the oligosaccharide subterminal structures rather than with the degree of sialylation of these oligosaccharides (18).

The possible participation of subterminal galactosyl residues in gp120/140–LN interaction was assessed by sequential treatment of WGA-eluted material with neuraminidase and β-galactosidase. In these conditions, there was a further band shift of 4 kDa in addition to that observed by neuraminidase treatment only, as detected by reaction of the polypeptide with the anti-β1-chain antiserum (Fig. 2*A*, lane G). No effect was noted in LN binding to the modified receptor (lane C).

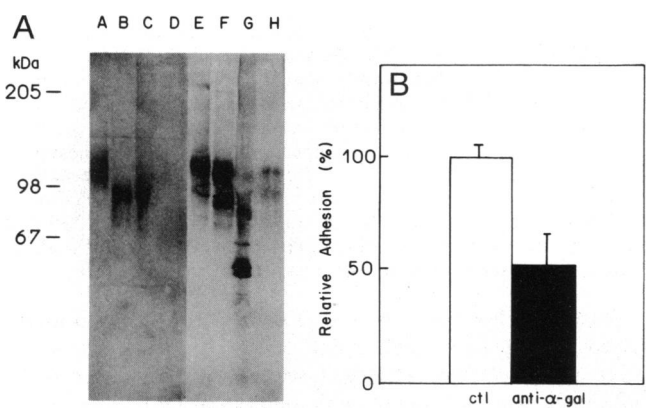

FIG. 2. Role of gp120/140 terminal sugar residues in its interaction with LN. (*A*) gp120/140-enriched cell extract, obtained from solid tumors, was digested with exoglycosidases (lanes A–D); shift analysis of exoglycosidase-treated material was performed with an antiserum that recognizes the β1 subunit of the receptor (lanes E–H). Protein separation was made under nonreducing conditions. Lanes A and E, undigested control, indicating the comigrating bands (lane A, laminin-binding polypeptide; lane E, β1-chain polypeptide). Lanes B and F, neuraminidase-treated material, indicating that asialo-gp120/140 was still able to bind LN. Lanes C and G, neuraminidase- and β-galactosidase-treated material, showing that subterminal β-galactoside residues are not involved in gp120/140 LN-binding activity. Lanes D and H, α-galactosidase-treated material, indicating that α-galactosyl residues present in gp120/140 play a role in its interaction with LN. (*B*) B16-F10 adhesion to LN-coated tissue culture wells could be inhibited by incubating cells with affinity-purified anti-α-galactosyl IgG. Control (ctl) incubation was performed with purified human IgG (from a pool of human serum). Maximal adhesion (considered as 100% of relative adhesion) was ≈5 × 10^4 cells. Bars represent means of triplicate values; standard deviations are indicated.

Conversely, on treating gp120/140 with α-galactosidase, we could abolish its LN-binding activity (Fig. 2*A*, lane D). In addition to the shift in molecular mass of the β1-chain polypeptide (lane H), α-galactosidase digestion was also monitored by using either affinity-purified anti-α-galactosyl IgG or biotin-labeled BSA in conventional Western blots (data not shown).

B16-F10 cell adhesion to LN-coated surfaces was also impaired by anti-α-galactosyl IgG, confirming the role of α-galactoside-bearing proteins in cell–matrix interactions (Fig. 2*B*). Penno and colleagues (19) have shown that terminal galactosylation of a 110-kDa cell-surface glycoprotein is correlated with invasiveness of murine adrenal carcinoma cells and their adherence to LN. In human models, adhesion of breast cancer cell lines to LN or to human umbilical vein was inhibited by antibodies against αGal(1–3)Gal structures (20). Our results suggest a possible molecular mechanism for the interaction between LN and integrin based on recognition of receptor α-galactosyl residues.

Laminin Binds Preferentially to the Integrin α Subunit. As depicted in Fig. 3, it was possible to separate the comigrating LN-binding polypeptide from the β1 integrin chain, according to their respective isoelectric points. The polypeptide recognized by the anti-β1 integrin antiserum had a pI of 4.3–4.8, whereas the LN binding protein presented a pI of 5.0–5.4, consistent with that ascribed to the α6 subunit (21). Attempts were made to identify positively the LN binding polypeptide by using different anti-α6 antibodies; however, none of them was suitable for Western blots in murine models. The carbohydrate-dependent interaction between ligand and receptor postulated here may explain why the α subunit alone continued to function as a laminin receptor *in vitro*, even after exposure to detergents and denaturants.

gp120/140 Is a L-PHA-Reactive Glycoprotein. gp120/140 was recognized by L-PHA, indicating that this glycoprotein bears β1–6-branched *N*-acetyllactosamine structures in tri- and tetraantennary complexes. Studies with tri- and tetraantennary minimal energy conformers indicate that the β1–6 branch is folded back to the protein structure (22). This

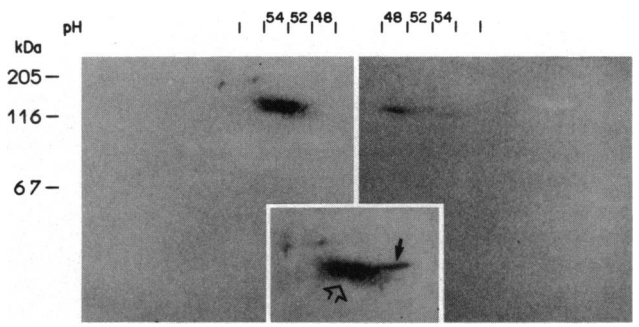

FIG. 3. Laminin binds preferentially to the integrin α chain. gp120/140-containing material was separated on two-dimensional SDS/polyacrylamide gel under nonreducing conditions and transferred onto nitrocellulose. (*Left*) LN binding activity of a polypeptide whose estimated pI was 5.0–5.4. A comigrating band as far as molecular mass is concerned was recognized by an antiserum raised against the β1-chain polypeptide (*Right*); however, the pI of this polypeptide was in the 4.3–4.8 range. (*Inset*) The same filter as in *Left* developed sequentially for LN-binding proteins (open arrowhead) and then for β1-chain polypeptide (solid arrow), denoting that LN bound preferentially to the integrin α subunit. Note the panels are displayed in reverse orientation.

oligosaccharide could be a likely modulator of protein conformation and, thus, a putative modifier of protein function. Synthesis of this branch is dependent on *N*-acetylglucosaminyltransferase V activity (GlcNAc-T V) (23). SW, a mannosidase II inhibitor, leads to accumulation of improper substrates for GlcNAc-T V, thus inhibiting indirectly the synthesis of β1–6-branched oligosaccharides (24). We have analyzed a possible functional role for L-PHA-reactive oligosaccharides in gp120/140.

Fig. 4 depicts the effect of SW on some features of B16-F10 cells, related to LN-binding activity (lanes A and B), expression of β1-chain glycoforms (lanes C and D), and L-PHA reactivity (lanes E and F). Lanes A, C, and E show extracts of control B16-F10 cells; lanes B, D, and F represent extracts of SW-treated B16-F10 cells. The efficiency of SW treatment was ascertained by loss of L-PHA reactivity. gp120/140 is one of the major L-PHA-reactive glycoproteins present in B16-F10 cell extracts (lane E) comigrating with polypeptides recognized by the anti-β1-chain antiserum (lane C). The β1-chain polypeptide, which presented the smaller apparent molecular mass, is an immature form of the other (pre-β1). No effect was observed either on gp120/140 binding to LN or on its electrophoretic mobility after SW treatment (lanes A and B). The band shift observed for the β1-chain polypeptide indicated clearly that this chain, and not the α subunit, bears L-PHA-reactive oligosaccharides.

To evaluate the functional role of β1–6-branched oligosaccharides in the β1 chain, it was necessary to ascertain that SW treatment did not interfere with the sorting of gp120/140 to the plasma membrane. ELISAs using nonpermeabilized cells that adhered to the solid phase were performed by using L-PHA, GoH3 (anti-α6-chain antibody), and anti-β1 antiserum as probes. Under these conditions, it was possible to assess only those polypeptides expressed at the cell surface. No significant variation, as analyzed by Student's *t* test, was observed regarding either α6- or β1-chain expression (Fig. 5*A*). Therefore, SW-treated B16-F10 cells express equal amounts of α6 and β1 integrin chains at the cell surface, when compared to the control group, in spite of being underglycosylated. We could then assess the functional role of L-PHA-negative β1 chains on B16-F10 adhesion properties.

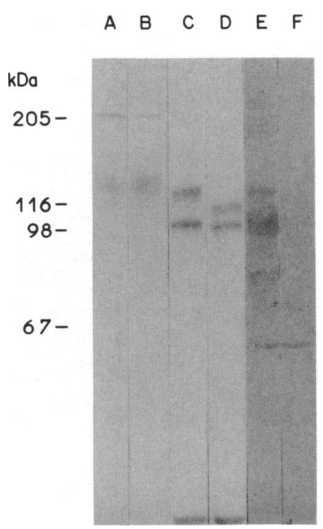

FIG. 4. SW effect on gp120/140 laminin binding activity. Control and SW-treated B16-F10 cells were compared regarding LN binding activity (lanes: A, control; B, SW treated), β1-chain polypeptide glycoforms (lanes: C, control; D, SW treated), and L-PHA reactivity (lanes: E, control; F, SW treated). gp120/140 is one of the major L-PHA-reactive glycoproteins from B16-F10 cells (lane E); SW impaired the synthesis of complex glycans responsible for such reactivity (lane F).

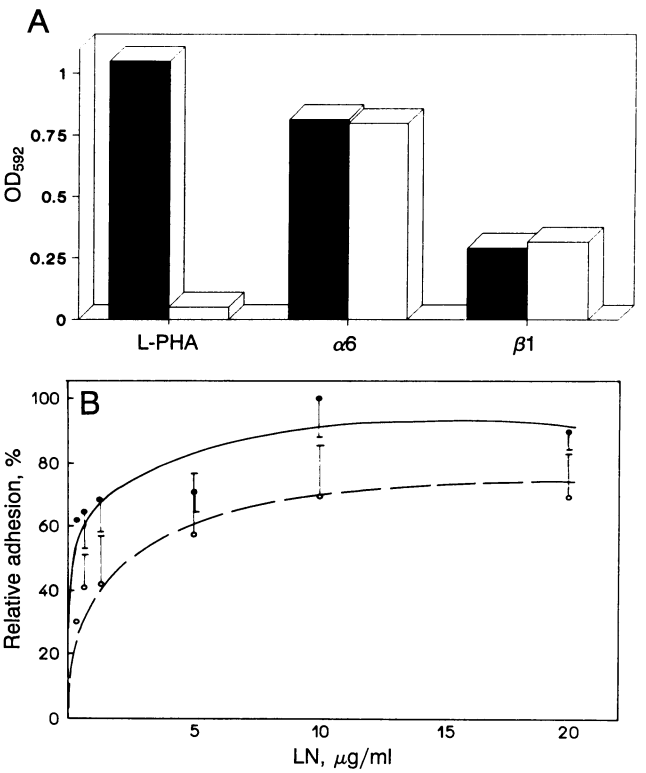

FIG. 5. SW does not impair either integrin chain sorting to the plasma membrane or cell adhesion on LN substrata. (*A*) ELISAs on nonpermeabilized fixed cells were done to assess α6 and β1 chains at the cell surface upon SW treatment. Reactivity to L-PHA ascertained the treatment efficiency. Bars represent means of three independent assays performed in triplicate. No significant differences were observed for α6 and β1 expression between control (■) and SW-treated (□) cells. (*B*) Metabolically labeled B16-F10 cells (control and SW treated) were plated on LN-coated tissue culture wells (range, 0.325–20 μg per ml per well). Adherent cells were assayed with a liquid scintillation counter. Solid circles represent adhesion of B16-F10 control cells; open circles represent adhesion of SW-treated cells. Each point represents the mean of five experiments. SDs are indicated. Cells adhered maximally at a LN concentration of 10 μg/ml (≈10^5 cells); this point was considered 100% and was used for estimating relative adhesion over the range studied. Differences observed did not reach statistical significance.

Spreading, but Not Adhesion, Is Associated with β1-Subunit Glycosylation. Although a small decrease of B16-F10 adhesiveness to LN was observed after SW treatment, such a difference was not statistically significant (Fig. 5*B*). However, the most striking SW effect was not on adhesion itself, but rather on B16-F10 spreading on LN, as illustrated in Fig. 6. Fig. 6 (*A* and *B*) shows B16-F10 adhesion to LN and FN, respectively. Fig. 6 (*C* and *D*) shows adhesion of treated B16-F10 cells to LN and FN, respectively. Spreading on LN, but not on FN, was considerably impaired in the presence of SW. Morphometric measurements indicated that SW-treated cells were significantly smaller than control cells (≈20% smaller). No significant difference was observed regarding FN adhesion.

SW-treated B16-F10 cells maintained both their ability to bind LN (Fig. 4, lane B) and their adhesiveness to LN-coated plates. It was possible, however, to impair their spreading ability by inhibiting the complete processing of the carbohydrate chains present at their cell surface, especially on the β1 chain. Arroyo *et al.* (25) have shown that cell spreading is a β1-chain-mediated effect. We have shown here that cell spreading may be associated with the glycosylation state of the β1 integrin chain.

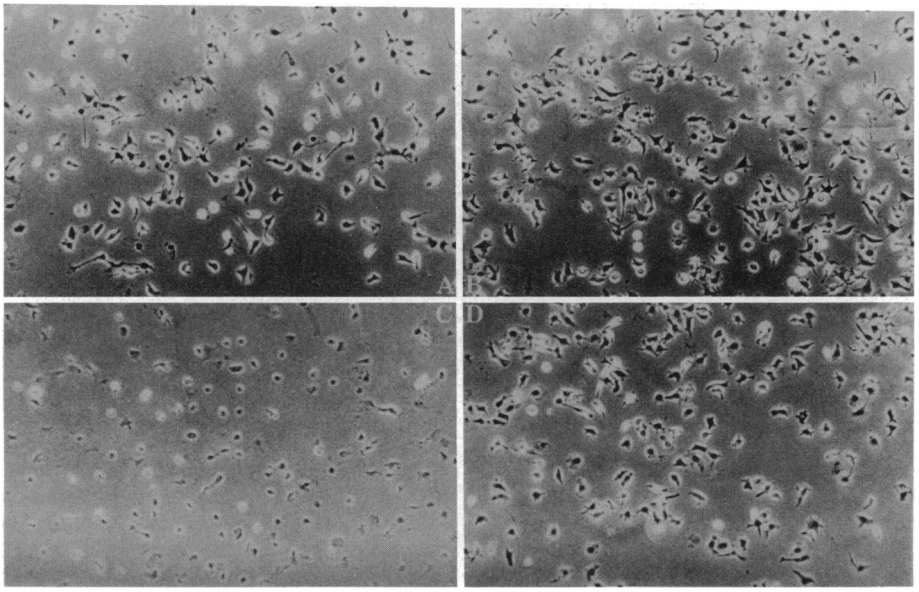

FIG. 6. SW effect on B16-F10 spreading on LN. B16-F10 spreading on LN-coated surfaces was analyzed morphometrically (*A*, control; *C*, SW treated). A significant decrease on spreading of SW-treated cells to LN was observed, whereas no effect was observed for spreading on FN-coated surfaces (*B*, control; *D*, SW treated). Morphometric measurements led to the observation that SW-treated cells are ≈20% smaller than control cells. Impaired spreading was associated with the expression of underglycosylated β1-chain polypeptides on B16-F10 cell surfaces.

Carbohydrate recognition has been implicated in a growing number of cellular functions (26). Assuming the integrin nature of gp120/140, we could show that LN bound preferentially to the α chain and that this interaction is dependent, at least in part, on terminal α-galactosyl residues. On the other hand, L-PHA-reactive oligosaccharides present in the β1 integrin chain are associated with spreading on LN. It is tempting to speculate that multiple affinity states of integrins, based on their glycosylation pattern, could modulate cell–matrix interactions.

We are grateful to Dr. S. Hakomori and Dr. U. Galili for helpful suggestions and to the investigators who provided us with antibodies. We thank N. Cella and Dr. L. L. Villa for critical review of the manuscript and L. C. B de Sousa for photographic work. R.C. and S.S.V. are fellows from Fundação de Amparo o Pesquisa do Estado de São Paulo (Grants 90/4529-2 and 89/3143-6, respectively).

1. Chammas, R. & Brentani, R. (1991) *Tumor Biol.* **12**, 309–320.
2. Humphries, M. J. (1990) *J. Cell Sci.* **97**, 585–592.
3. Chammas, R., Veiga, S. S., Line, S., Potocnjak, P. & Brentani, R. (1991) *J. Cell Biol.* **266**, 3349–3355.
4. Paullson, M., Aumailley, M., Deutzmann, R., Timpl, R., Beck, K. & Engel, J. (1987) *Eur. J. Biochem.* **166**, 11–19.
5. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857.
6. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
7. O'Farrell, P., Goodman, H. M. & O'Farrell, P. (1977) *Cell* **12**, 1133–1142.
8. Almeida, I., Milani, S., Gorin, A. & Travassos, L. (1991) *J. Immunol.* **146**, 2394–2400.
9. Engvall, E. (1980) *Methods Enzymol.* **70**, 419–439.
10. Knibbs, R., Goldstein, I., Ratcliffe, R. & Shibuya, N. (1991) *J. Biol. Chem.* **266**, 83–88.
11. Shibuya, N., Goldstein, I., van Damme, E. & Peumans, W. (1988) *J. Biol. Chem.* **263**, 728–734.
12. Cumming, R. & Kornfeld, S. (1982) *J. Biol. Chem.* **257**, 11230–11234.
13. Dennis, J., Waller, C. A. & Schirrmacher, V. (1984) *J. Cell Biol.* **99**, 1416–1423.
14. Sonnenberg, A., Jamssen, H., Hogervorst, F., Calafat, J. & Hilgers, J. (1987) *J. Biol. Chem.* **262**, 10376–10383.
15. Lloyd, K. O., Travassos, L. R., Takahashi, T. & Old, L. J. (1979) *J. Natl. Cancer Inst.* **63**, 623–634.
16. Morgenthaler, J., Kemmner, K. & Brossmer, R. (1990) *Biochem. Biophys. Res. Commun.* **171**, 860–866.
17. Acheson, A., Sunshine, J. & Rutishauser, U. (1991) *J. Cell Biol.* **114**, 143–153.
18. Passaniti, A. & Hart, G. (1988) *J. Biol. Chem.* **263**, 7591–7603.
19. Penno, M. B., Passaniti, A., Fridman, R., Hart, G., Jordan, C., Kumar, S. & Scott, A. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6057–6061.
20. Castronovo, V., Colin, C., Parent, B., Foidart, J. M., Lambotte, R. & Mahieu, P. (1989) *J. Natl. Cancer Inst.* **81**, 212–216.
21. Hemler, M. E., Crouse, C., Takada, Y. & Sonnenberg, A. (1988) *J. Biol. Chem.* **263**, 7660–7665.
22. Meyer, B. (1990) *Topics Curr. Chem.* **154**, 143–208.
23. Dennis, J. & Laferte, S. (1989) *Cancer Res.* **49**, 945–950.
24. Elbein, A. (1987) *Annu. Rev. Biochem.* **56**, 497–534.
25. Arroyo, A. G., Sanchez-Mateos, P., Campanero, M., Martin-Padura, I., Dejana, E. & Sanchez-Madrid, F. (1992) *J. Cell Biol.* **117**, 659–670.
26. Feizi, T. (1991) *Trends Biochem. Sci.* **16**, 84–86.